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14. ABSTRACT:

Purpose: Exercise is becoming readily accepted as a beneficial adjunct therapy to maintain or enhance quality of life in breast cancer patients during adjuvant chemotherapy. An essential precursor to these studies is to investigate whether exercise modulates the antitumor efficacy of chemotherapeutic agents.

Experimental Design: Athymic female mice were transplanted with MDA-MB-231 breast xenografts and randomly assigned to one of four groups (n= 21 per group): (i) control, (ii) exercise only, (iii) doxorubicin only, or (iv) exercise + doxorubicin. Exercise groups performed progressive treadmill running up to 18m/min at 0% grade for 45mins, 5 days/wk for 8 weeks.

Results: Tumor growth delay was significantly longer in the doxorubicin only and exercise + doxorubicin groups compared with the control (median 42 vs. 25 days, p=0.0082; 36 vs. 25 days, p=0.029, respectively) and exercise only groups (median 42 vs. 25 days, p=0.029; 36 vs. 25 days, p=0.080, respectively). There was no significant difference between doxorubicin only and exercise + doxorubicin groups (median 42 vs. 36 days, p=0.33) suggesting that moderate intensity exercise does not significantly influence doxorubicin-induced tumor growth delay.

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Table of Contents

INTRODUCTION

Several recent randomized trials have examined the role of exercise as a supportive intervention for breast cancer patients during conventional adjuvant chemotherapy (Mock, Pickett et al. 2001; Segal, Evans et al. 2001; Mock, Frangakis et al. 2004). Results of these trials have provided preliminary evidence that exercise training is a feasible and supportive intervention that may attenuate a broad range of deleterious symptoms (e.g., functional decline, fatigue, nausea) associated with cytotoxic therapy, leading to clinically relevant improvements in patients quality of life (QoL) (Mock, Pickett et al. 2001; Segal, Evans et al. 2001; Mock, Frangakis et al. 2004). While the importance of QoL as a clinical end-point is clear, a critical and previously unexplored corollary to this line of investigation is whether exercise training influences the anticancer effects of conventional cytotoxic therapy. The potential interaction between exercise and chemotherapy efficacy is biologically plausible. Exercise is a potent pleiotropic intervention that influences a wide spectrum of biologic processes that could potentially modulate the cytotoxicity of chemotherapeutic agents. Indeed, prior preclinical studies have reported both an inhibitory (Cohen, Choi et al. 1988; Baracos 1989; Thompson, Westerlind et al. 1995; Davis, Kohut et al. 1998; Zielinski, Muenchow et al. 2004) and augmentary (Thompson, Ronan et al. 1988; Thompson, Ronan et al. 1989; Woods, Davis et al. 1994) effect of endurance exercise training on mammary tumor growth and progression, although others have reported no association (Hoffman-Goetz, May et al. 1994). To our knowledge however, no study has examined the potential interaction between exercise and concurrent administration of chemotherapy.

BODY

The following section describes the research accomplishments achieved to date associated with each tasks outlined in the approved statement of work.

Task 1: Obtain Ethical Approval for Study (Months 1-2)

The ethics application was originally submitted to the Cross Cancer Institute's (CCI) Animal Research Ethics Board on June 27, 2003. Unfortunately, we experienced a number of problems in attempting to secure ethical approval for this study. Although the project had received external funding from the U.S. Department of Defense Breast Cancer Research Program, the CCI Animal Research Ethics Board committee also wanted to obtain an external merit review of the project. In totality, the whole process took approximately five months and ethical approval was finally awarded in October, 2003. The process of obtaining Cross Cancer Institute ethical approval was far more complicated than we originally anticipated and significantly delayed the initiation of the project.

Task 2: Pilot Testing of Breast Carcinoma Cell Lines and Anthracycline-Based Chemotherapy (Months 2-4)

After obtaining ethical approval we initiated the first of two planned pilot studies investigating the growth rate of breast carcinoma cell line MDA-MB 231. To confirm the tumorgenicity of MDA-MB 231 human breast carcinomas under our laboratory conditions we initiated a pilot study using 18 athymic female mice. Cells were harvested in the exponential growth phase and were stained with Trypan Blue and the number of viable cells was counted using a hemacytometer. Mice were then subcutaneously implanted with 1×10^6, 2×10^6, and 3×10^6 cells in the right flank of 6 mice per group (n=18 in total) anesthetized with Isoflurane. Mice were then observed to determine the number of animals to develop palpable tumors at 30 days and determine the average tumor volume. Tumor volume was measured in two dimensions with microcalipers using the following equation: $V = L \times W \times 0.5W$ where W is the width of the xenograft and L is the length as suggested by Bandyopadhyay et al. (Bandyopadhyay, Lopez-

Casillas et al. 2002), Qin et al. (Qin, Shao et al. 2002) and Trial et al. (Trail, Willner et al. 1999). If less than 4 animals have palpable tumors at 30 days, the number of cells was to be increased and the experiment repeated using 6 athymic female mice. Unexpectedly, breast carcinoma cell line dosages of 1×10^6, 2×10^6, and 3×10^6 cells generally failed to produce palpable tumors at 30 days in athymic female mice. After consultation with my team and other investigators at our institution (Cross Cancer Institute) with prior experience with the breast carcinoma cell line MDA-MB 231, we concluded that we were satisfied with the viability of the cells and therefore opted to increase the cell line dosage before locating an alternative source of cells. As such, we repeated the experiment, however this time injecting mice subcutaneously with 5×10^6 cells. This cell line dosage produced palpable tumors by 30 days in all mice. Therefore, we concluded that these tumor cells were viable and this dosage was used for subsequent experiments.

Following the completion of the first pilot study as described, we initiated the second pilot study to determine the appropriate dose and schedule of Adriamycin in athymic female mice bearing MDA-MB 231 human breast carcinoma cell line. In previous reports, athymic female mice bearing the MDA-MB 231 breast carcinoma cell line have been treated with various doses and schedules of Adriamycin (e.g., 5mg/kg every four days and 5mg/kg every 7 days intravenously in a lateral tail vein and 5mg/kg every 5 days representing the highest tolerable doses and reflecting current clinical therapy guidelines). Although these protocols were effective at significantly reducing tumor volume in MDA-MB 231 breast carcinoma bearing mice, animals also experienced weight loss (approaching 10% of initial weight by 2 weeks) indicating that maximum tolerated dosage was met or exceeded. Because exercise may also produce weight loss, we performed a second pilot study with athymic mice bearing the MDA-MB 231 human breast carcinoma cell line to determine an appropriate dose and schedule of Adriamycin to produce a 5-7.5% weight loss. Twelve experimental animals were purchased at 21 days of age and allowed to acclimatize for 10 days prior to the commencement of pilot study two. At 31 days of age, MDA-MB-231 carcinoma cells (5×10^6 cells prepared from donor tumors) were

subcutaneously implanted into the right flank of all animals. By 38 days all experimental animals had palpable tumors and were subsequently randomly assigned to receive the following doses: 3mg/kg (Group 1), 4 mg/kg (Group 2), 5mg/kg (Group 3), and 6mg/kg (Group 4) every 7 days for 8 weeks (Hardman, Avula et al. 2001). Injection sites were rotated to minimize local tissue irritation and injury. Adriamycin timing was consistent for all animals. The results of the second pilot study are provided below.

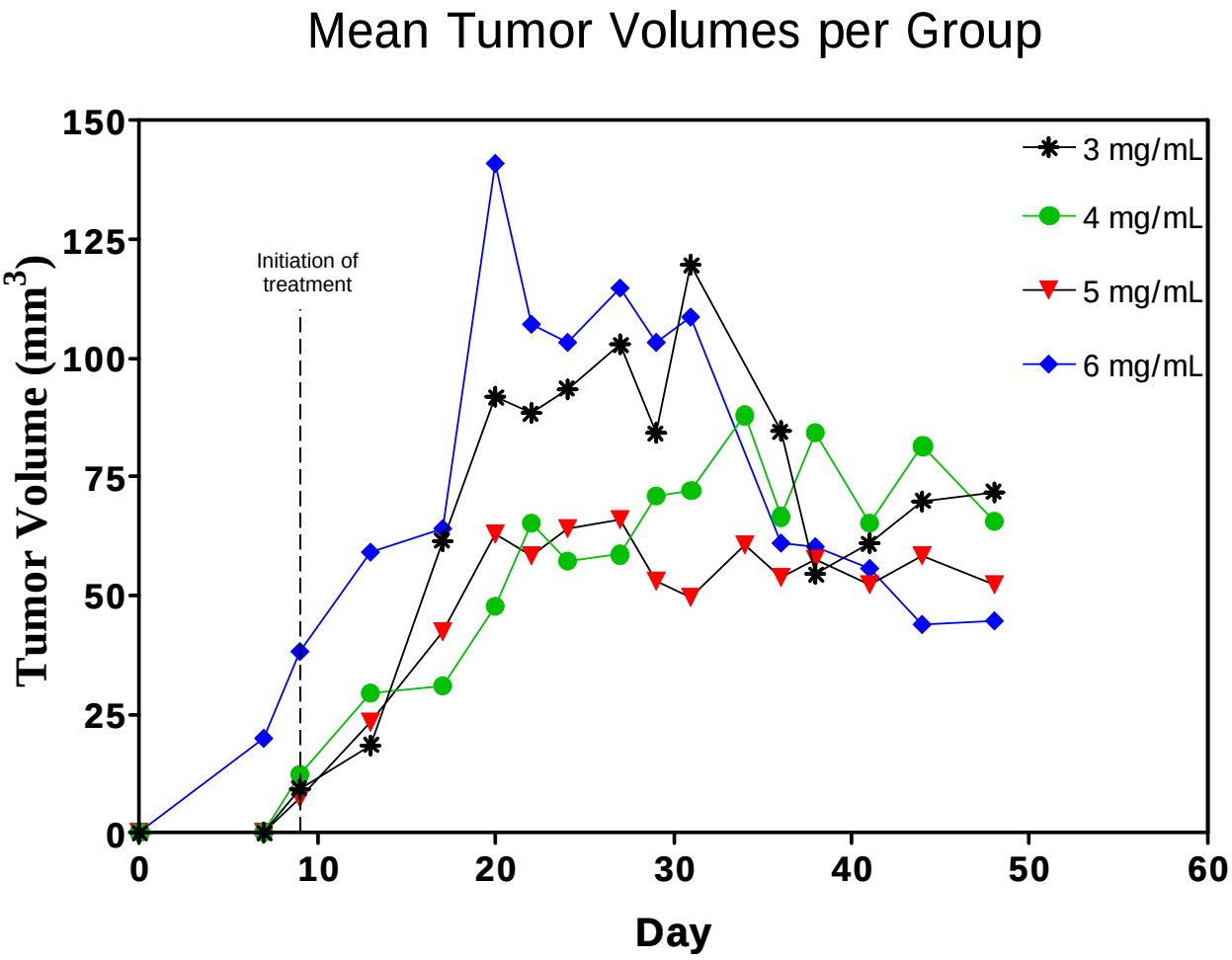

Figure 1. Determining the appropriate dose and schedule of Adriamycin in athymic female mice bearing MDA-MB 231 human breast carcinoma cell line (5 x 10^6). Twelve mice were randomly assigned into four groups of three. ✱ 3 mg/kg every 7 days, ● 4 mg/kg every 7 days, ✡ 5 mg/kg every 7 days ■ 6 mg/kg every 7 days. Tumor growth after initiation of Adriamycin treatment. By Day 7 mice in all groups had palpable tumors. Tumor volumes rapidly increase in all groups until approximately day 20 onwards when Adriamycin administration begins to check the tumor growth rate in all groups. Close observation of the tumor growth rates reveals that Adriamycin dosage in Group 1 (3 mg/kg) provided adequate tumor growth until approximately Day 39 where tumor volumes rapidly increase, suggesting tumors had become

resistant to this level of Adriamycin dosage. Group 4 (6 mg/kg) also provides adequate tumor growth control, however from approximately Day 27 onwards tumor volumes continually decrease, suggesting that these tumors may eventually become immeasurable. Finally, Groups 2 (4 mg/kg) and 3 (5 mg/kg) both provide adequate tumor growth control. Mice were sacrificed when tumor volume reached 1100mm^3 as required by institutional guidelines. Statistical analysis of the mean tumor growth rates were not performed because we were interested in which drug dosage provided controllable tumor growth control across the entire study rather than which drug dosage produced the lowest tumor volume at the end of the study.

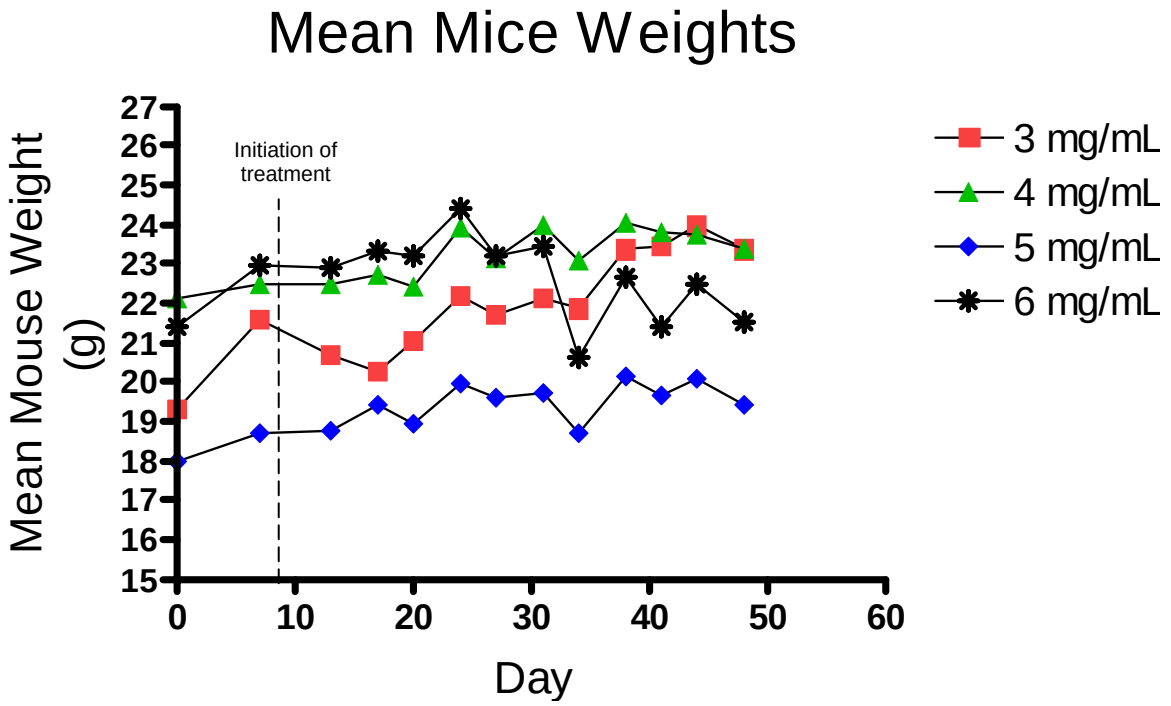

Figure 2. Determining the appropriate dose and schedule of Adriamycin in athymic female mice bearing MDA-MB 231 human breast carcinoma cell line (5 x 10^6). Twelve mice were randomly assigned into four groups of three. ✸ 3 mg/kg every 7 days, ● 4 mg/kg every 7 days, ✡ 5 mg/kg every 7 days ■ 6 mg/kg every 7 days. Mean mice weights after initiation of Adriamycin treatment. By Day 7 mice in all groups had palpable tumors. Close observation indicates minimal mean mice body weight loss in all groups over the entire course of the experiment, except in Group 4 (6 mg/kg) where mean mice body weight rapidly decrease from approximately Day 30 onwards suggesting that that maximum tolerated dose has been reached or exceeded.

Taking into consideration the mean tumor volumes (Figure 1) and the mean mice body weights per group (Figure 2) we concluded that that 4mg/kg every 7 days (Group 2) was the appropriate dose of Adriamycin. In Group 1 (3mg/kg), the drug dosage was well tolerated however this dosage did not provide adequate controllable tumor cell growth. Similarly, in Group

4 (5 mg/kg) the drug dosage was also well tolerated, however from approximately Day 27 onwards tumor volumes continually decreased and we were concerned that these tumors may eventually become immeasurable. In addition, 4 mg/kg was providing adequate tumor growth control, so subjecting mice to higher doses of Adriamycin (5 mg/kg) and possibly higher toxicity to achieve similar tumor control seemed inane. Finally, in Group 4 (6 mg/kg) the drug dosage was not well tolerated, mice lost weight from approximately Day 30 onwards suggesting that maximum tolerated dose has been reached or exceeded. Again, similar to Group 3, from approximately Day 19 onwards, tumor volumes continually decreased and we were again concerned that these tumors may eventually become immeasurable. Based on this evidence, 4 mg/kg was selected as the appropriate dosage of Adriamycin. Importantly, several previous reports examining the pharmacokinetic profile of Adriamycin in athymic mice (Giuliani, Zirvi et al. 1981; Wassermann and Rasmussen 1987; Inaba, Kobayashi et al. 1989; Kubota, Inoue et al. 1993), have demonstrated that similar dosing schedules produced tumor growth delay and statistically significant decrease in mean tumor volume with acceptable toxicity. Furthermore, the pharmacokinetic profile of doxorubicin in athymic mice has been shown to mimic clinical pharmacokinetics and pharmacodynamics of anticancer drugs in the clinical setting (Kubota, Inoue et al. 1993).

In the interest of using the lowest possible number of mice, we did not include a positive control group in the second pilot study. We realize that without an untreated control it is difficult to conclude whether different dosages of Adriamycin differential influence tumor growth however, our results certainly suggest that all selected doses of Adriamycin produced some form of controllable tumor growth (see Figure 1). Our observation that the tumor volume growth curves in pilot study one were higher with the same breast carcinoma cell line dosage over the same number of days in comparison to pilot study two supports this notion. In addition, the main experiment does include a positive control group therefore we will be able to definitely conclude

from this on-going experiment if 4 mg/kg of Adriamycin every 7 days does influence tumor growth.

Task 3: Confirm Testing/Intervention Protocol (Months 4-5)

a. Clarify exercise training, breast carcinoma cell line administration, and drug dosage protocol

Following the completion of the pilot studies (as described), we wanted to confirm all experimental procedures before initiating the final protocol. The results of pilot study one indicated that the breast carcinoma cell line dosage of 5×10^6 cells produced palpable tumors by 30 days and was appropriate for the study. The results of pilot study two indicated that 4mg/kg of doxorubicin every 7 days produced controllable tumor growth without significant weight loss and was also deemed appropriate for the main study. Regarding the exercise training protocol, based on prior investigations we had proposed to progressively train animals to run at 22m/min at 0% grade for 45 minutes 5 days/week for 8 weeks. However, when applying this protocol in our laboratory settings, animals were unable to adequately run at this speed. As such, we reduced the running speed to 18m/min. All other exercise prescription parameters remained the same.

b. Clarify necropsy and histological procedures

Forty-eight hours following the final exercise training session, all experimental animals will be sacrificed via carbon dioxide. The primary tumor will be surgically removed, weighed and histologically processed. The lungs will be removed and examined visually as well as microscopically for the formation of tumor cell colonies. Additional tissues such as a skeletal muscle, the heart and the liver and blood will also be removed at necropsy. These tissues will be individually placed in i) a labeled vial and flash frozen with liquid nitrogen, and ii) formalin fixed for future analysis. The animals will also be sacrificed by CO_2 and the tumors dissected and processed as above if any of the following conditions are met:

- if a tumor is beginning to corrode through the skin

- if a tumor is inhibiting the natural movement of the animal
- if a tumor is causing irritation to the animal
- if a tumor measures 1.0cm x 1.0cm or greater
- if feet develop abrasions
- if mice are injured

All procedures will be performed at the Cross Cancer Institute under the direction of John Mackey. Histopathology of necropsy specimens will be performed by Dr. Chiu, University of Alberta Hospital, Department of Pathology. Suspected infection-associated animal mortalities will be thoroughly investigated via a complete post-mortem dissection in Health Services Laboratory Animal Services (HSLAS) at the University of Alberta. Specifically, the necropsy form will be completed and Drs. Nation and Uwiera (HSLAS pathologists) will be immediately contacted and de-briefed regarding the animal mortality. Drs. Nation and Uwiera will then perform a complete dissection of the animal including a bacterial culture on the gastrointestinal contents to identify any abnormal bacteria and a complete analysis of the animals' food so comparison bacteria analyses can be performed.

c. Ensure all necessary equipment (e.g., drugs, cell lines, etc) is purchased and/or operational

All equipment and supplies have been purchased and are operational.

Task 4: Data Collection (Months 5-9)

a. Sixty athymic Fischer 344 mice will be purchased at 21 days of age

To increase the sample size and power we purchased 92 rather than 60 athymic mice. We also wanted to increase the number of purchased mice given that an injection of MDA-MB 231 at 5 x 10^6 does not form solid tumors in all recipient animals.

b. All animals will be allowed to acclimatize for 5 days prior to commencement of study-related procedures

Animals were allowed to acclimatize for 5 days prior to initiation of study-related procedures.

c. At 26 days of age MDA-MB-231 (at the designated dosage, prepared from a brei of donor tumors) will be subcutaneously implanted into the right flank of animals

MDA-MB-231 breast carcinoma cells (ATCC, Rockville, MD) (prepared from donor animals at 5 x 10^6) were subcutaneously implanted into the right flank of 92 female athymic Nude-nu mice (Harlan, WI) aged 3-4 weeks. Animals in which tumors failed to grow were excluded from the study (n=8). All animals were fed a modified basal diet (Harlan Teklad, WI) with 40% of calories from fat to reflect a typical North American diet (Birt, Copenhaver et al. 1997) and water *ad libitum*. The diet was freshly prepared weekly to prevent the fat from becoming rancid.

d. At 40 days of age, animals will be stratified and randomly assigned to one of four groups: (a) exercise alone, (b) chemotherapy alone, (c) exercise plus chemotherapy, and (d) control

Following tumor establishment (14 days, tumor volumes ~ 300 mm^3), mice were stratified by body weight and tumor volume and randomly assigned to one of four groups (n=21 per group): (i) control group ii) exercise only, (iii) doxorubicin only, or (iv) exercise + doxorubicin.

e. Based on the results of the second pilot study, animals assigned to chemotherapy alone and chemotherapy plus exercise will also receive intravenous injections of doxorubicin at the designated dosage

Doxorubicin (Adriamycin hydrochloride, Sigma Aldrich Canada Ltd, Oakville, ON) was administered via weekly intravenous lateral tail vein injections at 4mg/kg for 8 weeks. Injection sites were rotated to minimize local tissue irritation and injury.

f. The exercise groups will be progressively trained to run at 22m/min at 0% grade for 45 minutes 5 days/week for 8 weeks

Exercise groups performed progressive treadmill running up to 18m/min at 0% grade for 45 min, 5d.wk for 8 weeks. Exercise training began at 10m/min, 0% grade, for 10 minutes for 5 days/week in weeks 1 and 2 and was systematically increased until the desired exercise protocol was achieved. This training intensity corresponds to approximately 70-75% of murine maximal oxygen uptake (Fernando, Bonen et al. 1993). Electrical stimulation was not used to encourage the animals to run. To ensure similar physical and social environments, a second

treadmill was used as a sham exercise for the non-exercising groups (Thompson 1997). Tumor volume and body weight were measured twice weekly and animals were monitored continuously for the entire duration of exercise. Animal care was approved and in accordance with the Institutional Animal Care and Use Guidelines at the Cross Cancer Institute, Edmonton, Canada.

g. Forty eight hours after the final exercise session all experimental animals will be sacrificed. Primary tumors will be surgically removed, weighed and histologically processed

Mice were sacrificed when tumor volume reached 1100mm^3 as required by institutional guidelines or 48 hours following the final exercise training session. The primary tumor will be surgically removed, weighed and histologically processed. The lungs will be removed and examined visually as well as microscopically for the formation of tumor cell colonies. Additional tissues such as a skeletal muscle, the heart and the liver and blood will also be removed at necropsy. These tissues will be individually placed in i) a labeled vial and flash frozen with liquid nitrogen, and ii) formalin fixed for future analysis.

The primary endpoint was tumor growth delay, calculated as the number of days for each individual tumor to reach 1100mm^3. Tumor growth delay survival curves were analyzed using the Cox model for pairwise comparisons and relative risk estimates generated with 95% confidence intervals. The logrank test was used for the overall group comparison. Changes in body weight were analyzed using independent samples t-tests. Two-tailed tests were used for the analysis with a $p<0.05$ considered significant.

Task 5: Data Entry and Analysis and Manuscript Preparation and Submission (Months 10-12)

a. Data entry

Data entry was completed by a research assistant who was blinded to experimental groups and manipulations.

b. Statistical analysis of data from all aspects of the trial

All statistical analyses were performed by John Hanson, MS, who was also blinded to experimental group assignment.

c. Overview of the results

The preliminary results were overviewed by Drs. Jones, Mackey, Baracos, and Courneya. Tumor growth delay and between group comparisons are presented in Table 1. Tumor growth delay was significantly prolonged in the doxorubicin only and exercise + doxorubicin groups compared to the exercise only and control groups. There was no significant difference between doxorubicin only and exercise + doxorubicin groups or exercise only and control groups. At 45 days, Kaplan-Meier estimates indicated a 35% (95% CI=17% to 54%) survival rate for the doxorubicin only mice compared to 20% (95% CI=7% to 33 %) in the exercise + doxorubicin group, 16% (95% CI=2% to 31%) in the exercise only group and 0% in the control group (Figure 1). Body weight did not significantly change over the course of experiment in any group (Figure 2). All mice achieved the designated exercise protocol.

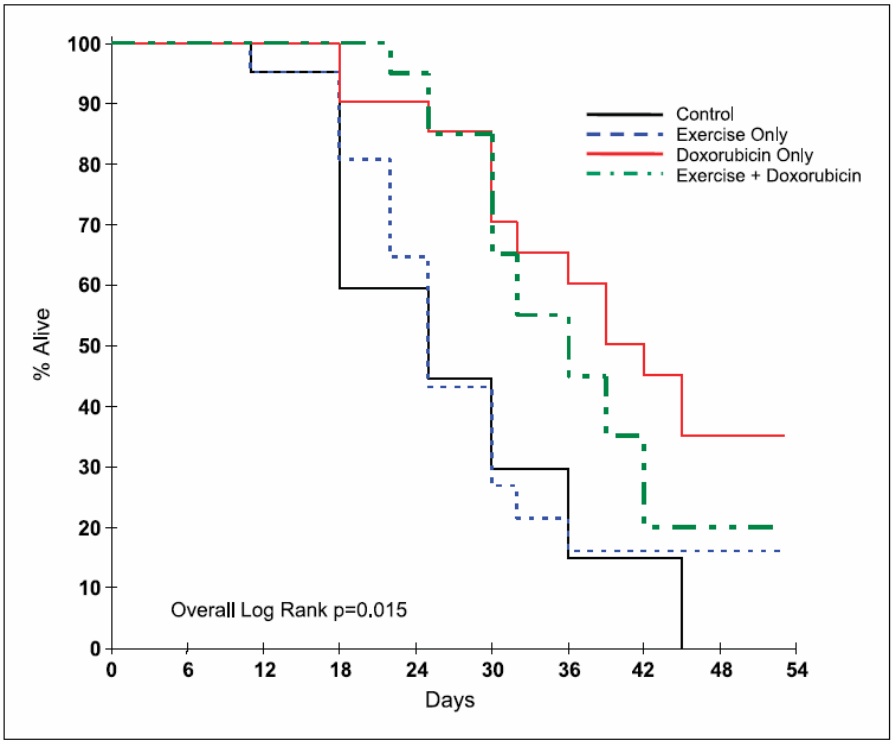

Fig. 1. Survival curves of athymic female mice implanted with MDA-MB-231 breast carcinoma xenografts. All animals were s.c. implanted with MDA-MB-231 breast carcinoma cells (5×10^6) in the right flank. Following tumor establishment (14 days, tumor volume ~300 mm^3), mice were stratified by body weight and tumor volume and randomly assigned to receive doxorubicin (4 mg/kg every 7 days), exercise training (18 m/min, 0% grade, 45 minutes, 5 d/wk for 8 weeks), doxorubicin + exercise or no intervention control. Tumor volume and body weight were measured twice weekly. Tumor growth delay was significantly prolonged in the doxorubicin-only and exercise + doxorubicin groups compared with the exercise-only and control groups (overall log rank, $P = 0.015$). There was no significant difference between doxorubicin-only and exercise + doxorubicin groups or exercise-only and control groups. At 45 days, Kaplan-Meier estimates indicated a 35% (95% CI, 17-54%) survival rate for the doxorubicin-only mice compared with 20% (95% CI, 7-33%) in the doxorubicin + exercise group, 16% (95% CI, 2-31%) in the exercise-only group and 0% in the control group.

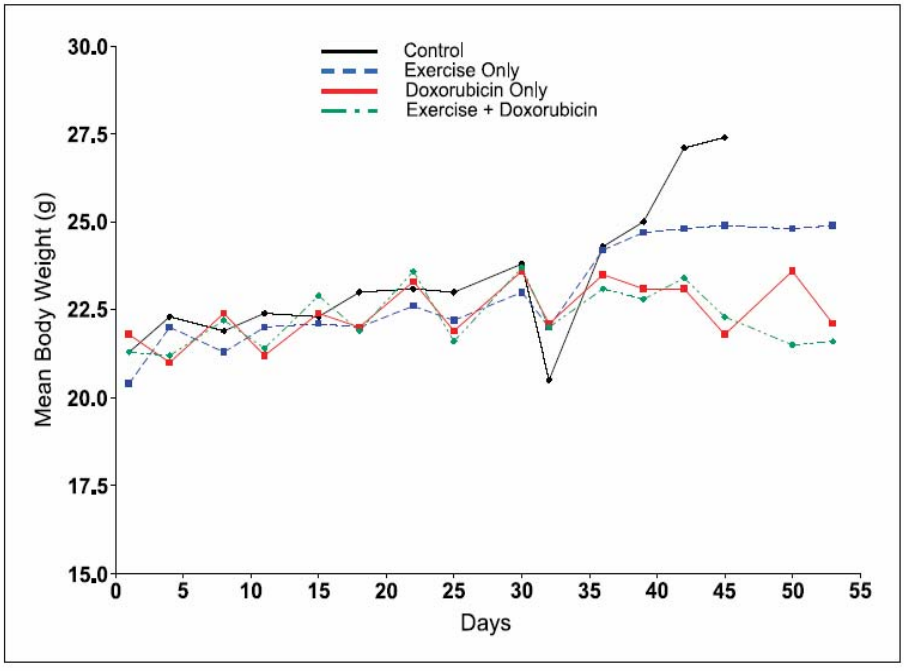

Fig. 2. Mean body weights (grams) of athymic female mice implanted with MDA-MB-231 breast carcinoma xenografts. All animals were s.c. implanted with MDA-MB-231 breast carcinoma cells (5×10^6) in the right flank. Following tumor establishment (14 days, tumor volume ~300 mm^3), mice were stratified by body weight and tumor volume and randomly assigned to receive doxorubicin (4 mg/kg every 7 days), exercise training (18 m/min, 0% grade, 45 minutes, 5 d/wk for 8 weeks), doxorubicin + exercise or no intervention control. Tumor volume and body weight were measured twice weekly. There were no significant differences in body weight between the groups over the course of the study ($P > 0.05$).

d. Final analysis of the results

Final data analyses were performed by John Hanson, MS as described.

e. Identification of appropriate journal(s)

Based on prior investigations of this nature, we selected *Clinical Cancer Research* as an appropriate outlet for this work.

f. Manuscript writing and preparation

The first draft of the manuscript was written and prepared by Dr. Jones. Substantial intellectual input into this draft was provided by Drs. Eves, Courneya, Chui, Baracos, and Mackey as well as Ms. Johnson and Mr. Hanson.

g. Manuscript submission

The manuscript was submitted to *Clinical Cancer Research* on April 4, 2005; revised June 2nd, 2005, and accepted for publication on June 15, 2005 (see Appendix A).

KEY RESEARCH ACCOMPLISHMENTS

- This is the first study to examine the potential interaction between exercise training and concurrent chemotherapy administration. Given the recent burgeoning number of studies examining the potential role of exercise as a supportive intervention for breast cancer patients during adjuvant or neoadjuvant therapy, these studies are essential to the interpretation and acceptance of exercise as a modifier of quality of life.
- Overall, the key finding of this experimental was that moderate intensity treadmill running did not significantly modulate doxorubicin-induced tumor growth delay in human breast cancer xenografts.
- These findings provide the necessary preliminary data for future studies to further investigate the effects of exercise training on the cytotoxic effects of chemotherapy and other conventional agents in the clinical setting.

REPORTABLE OUTCOMES

- Peer-reviewed manuscript publication in *Clinical Cancer Research* – the official journal of the American Association of Cancer Research (AACR). **Jones LW**, Eves ND, Courneya KS, Chiu BK, Baracos VE, Hanson J, Johnson L, Mackey JR. The effects of exercise training on antitumor efficacy of doxorubicin in MDA-MB-231 breast cancer xenografts. *Clinical Cancer Research*, 2005;11 6695-6698.
- Peer-reviewed abstract publication in the *Proceedings of the American Association of Cancer Research*. **Jones LW**, Eves ND, Courneya KS, Chiu BK, Baracos VE, Hanson J, Mackey JR. The effects of exercise training on antitumor efficacy of doxorubicin in MDA-MB-231 breast cancer xenografts. *Proceedings of the American Association of Cancer Research*, 2005;46.
- Poster presentation at the 96th Annual Meeting of the American Society of Cancer Research, April 16-20, Anaheim, CA.
- As part of this award, the PI (Dr. Jones) was able to complete his Postdoctoral Fellowship in Exercise Oncology at the University of Alberta and Cross Cancer Institute, Edmonton, AB, Canada.
- This award and subsequent publication significantly enhanced Dr. Jones' curriculum vitae and employment prospects. In fact, following completion of this project, Dr. Jones was recruited as an Assistant Professor in the Cancer Prevention, Detection, and Control Program within the Department of Medicine at Duke University Medical Center.
- This work directly supported a recent grant submission (by the PI) to the US Department of Defense Breast Cancer Research Program - Ideas award mechanism. The main purpose of this grant is to extend our current findings to the clinical setting to investigate the effects of exercise training on tumor vascularity and response to neoadjuvant anthracycline-containing chemotherapy for operable breast cancer: a phase II

randomized trial (BC050302). We are very pleased to report that this grant was approved for funding in December 2005 and the anticipated start date is April 1st, 2006.

- This award has provided an employment opportunity for a laboratory technician at the Cross Cancer Institute, Edmonton, Canada. In addition, this award has also allowed the technician to attend several training courses on mouse handling and necropsy.

- This award has also provided a unique training experience for an undergraduate student from the University of Alberta. This student has also attended several training courses that were supported by this award. Finally, involvement with this project significantly facilitated her recent successful admission to medical school at the University of Alberta.

CONCLUSION

"So what, who cares?"

The American Cancer Society recently published guidelines recommending that all cancer patients be encouraged to exercise during chemotherapy. These recommendations are primarily based on the promising preliminary evidence of the effects of exercise on maintaining or enhancing QoL (Brown, Byers et al. 2003). As such, studies investigating the potential interaction between exercise and chemotherapy efficacy are essential to the interpretation and acceptance of exercise as a modifier of QoL. As expected, groups that received doxorubicin had significantly prolonged tumor growth delay than groups who did not receive cytotoxic therapy. However, the key finding of this investigation was that moderate intensity treadmill running did not significantly modulate doxorubicin-induced tumor growth delay in MDA-MB-231 breast carcinoma xenografts.

A considerable number of exercise trials and preclinical studies in oncology have provided indirect evidence of the potential biologic mechanisms that may underlie the complex and multifaceted interaction between exercise, the tumor, and the antineoplastic effects of anthracycline-based chemotherapy. These biologic mechanisms include, but are not limited to exercise-modulated changes in hormonal (McTiernan, Tworoger et al. 2004) and metabolic profile (Fairey, Courneya et al. 2003), nitric oxide mediated peripheral blood flow (Hambrecht, Hilbrich et al. 2000; Hambrecht, Wolf et al. 2000), angiogenesis (Kraus, Stallings et al. 2004; Waters, Rotevatn et al. 2004), endogenous antioxidant expression (Ji 2002), and pharmacokinetic profile of agents (van Baak 1990). However, the present findings provide preliminary evidence that an exercise training program reflective of national exercise guidelines for cancer prevention and cardiovascular health (Pate, Pratt et al. 1995) in humans may not sufficiently perturb these pathways to interact with the anticancer effects of doxorubicin on breast carcinoma xenografts.

Several prior preclinical reports have demonstrated an inhibitory effect of exercise training on spontaneous and chemically-induced tumor growth, metastatic progression and microvessel density without concurrent chemotherapy (Baracos 1989; Thompson 1994; Thompson, Westerlind et al. 1995; Westerlind, McCarty et al. 2003; Zielinski, Muenchow et al. 2004). However, almost all of these studies have examined the effects of exhaustive treadmill running at approximately 80-90% of maximal oxygen consumption (VO_{2max}). These previous findings in combination with the present results suggest that exercise training needs to be performed at a high intensity to activate tumor modulating pathways (>70% VO_{2max}) (Thompson 1994). Support for this notion is provided by the fact that the tumor growth curves for the exercise only and control groups were essentially identical in the present study (see Figure 1). Of course, from a clinical perspective, it is unlikely that many breast cancer patients undergoing cytotoxic therapy will be able or willing to exercise at 80-90% VO_{2max}. Thus, further research is required to investigate if a dose-response relationship exists between exercise and cancer progression during concurrent antineoplastic therapy.

Obviously, several important differences exist between our mouse model of breast cancer and breast cancer patients in the clinical setting. First, mice in the present study were only a few weeks post weaning and thus, immature. Second, hormonal and immune profile of the groups is not equivalent. This may be particularly important given the potential biologic interaction between exercise, immune/hormonal profile and breast cancer prognosis (28). Finally, our breast cancer cell-line was implanted subcutaneously rather than at the relevant orthotopic site (i.e, mammary pad). Thus, caution must be taken when extrapolating the present results to women undergoing cytotoxic therapy for early-stage or metastatic breast cancer.

To summarize, the present results suggest that exercise training does not significantly modulate the antitumor efficacy of doxorubicin in breast cancer xenografts. Further studies investigating the effects of exercise on the cytotoxic effects of chemotherapy and other

conventional agents in the clinical setting are warranted. Such studies are essential to fully understand the safety and application of exercise as a supportive intervention in cancer control.

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APPENDIX I

Effects of Exercise Training on Antitumor Efficacy of Doxorubicin in MDA-MB-231 Breast Cancer Xenografts

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Key Words: Exercise, Breast Cancer Xenografts, Doxorubicin, Efficacy

Abstract

Purpose: Exercise is becoming readily accepted as a beneficial adjunct therapy to maintain or enhance quality of life in breast cancer patients during adjuvant chemotherapy. An essential precursor to these studies is to investigate whether exercise modulates the antitumor efficacy of chemotherapeutic agents.

Experimental Design: Athymic female mice were transplanted with MDA-MB-231 breast xenografts and randomly assigned to one of four groups (n= 21 per group): (i) control, (ii) exercise only, (iii) doxorubicin only, or (iv) exercise + doxorubicin. Exercise groups performed progressive treadmill running up to 18m/min at 0% grade for 45mins, 5 days/wk for 8 weeks.

Results: Tumor growth delay was significantly longer in the doxorubicin only and exercise + doxorubicin groups compared with the control (median 42 vs. 25 days, p=0.0082; 36 vs. 25 days, p=0.029, respectively) and exercise only groups (median 42 vs. 25 days, p=0.029; 36 vs. 25 days, p=0.080, respectively). There was no significant difference between doxorubicin only and exercise + doxorubicin groups (median 42 vs. 36 days, p=0.33) suggesting that moderate intensity exercise does not significantly influence doxorubicin-induced tumor growth delay.

Conclusion: These studies are essential to fully understand the safety and application of exercise as a supportive intervention in cancer control.

Introduction

Several recent randomized trials have examined the role of exercise as a supportive intervention for breast cancer patients during conventional adjuvant chemotherapy (Mock, Pickett et al. 2001; Segal, Evans et al. 2001; Mock, Frangakis et al. 2004). Results of these trials have provided preliminary evidence that exercise training is a feasible and supportive intervention that may attenuate a broad range of deleterious symptoms (e.g., functional decline, fatigue, nausea) associated with cytotoxic therapy, leading to clinically relevant improvements in patients quality of life (QoL) (Mock, Pickett et al. 2001; Segal, Evans et al. 2001; Mock, Frangakis et al. 2004). While the importance of QoL as a clinical end-point is clear, a critical and previously unexplored corollary to this line of investigation is whether exercise training influences the anticancer effects of conventional cytotoxic therapy. The potential interaction between exercise and chemotherapy efficacy is biologically plausible. Exercise is a potent pleiotropic intervention that influences a wide spectrum of biologic processes that could potentially modulate the cytotoxicity of chemotherapeutic agents. Indeed, prior preclinical studies have reported both an inhibitory (Cohen, Choi et al. 1988; Baracos 1989; Thompson, Westerlind et al. 1995; Davis, Kohut et al. 1998; Zielinski, Muenchow et al. 2004) and augmentary (Thompson, Ronan et al. 1988; Thompson, Ronan et al. 1989; Woods, Davis et al. 1994) effect of endurance exercise training on mammary tumor growth and progression, although others have reported no association (Hoffman-Goetz, May et al. 1994). To our knowledge however, no study has examined the potential interaction between exercise and concurrent administration of chemotherapy.

Materials and Methods

MDA-MB-231 breast carcinoma cells (ATCC, Rockville, MD) (prepared from donor animals at 5 x 10^6) were subcutaneously implanted into the right flank of 92 female athymic Nude-nu mice (Harlan, WI) aged 3-4 weeks. Animals in which tumors failed to grow were excluded from the study (n=8). All animals were fed a modified basal diet (Harlan Teklad, WI) with 40% of calories from fat to reflect a typical North American diet (Birt, Copenhaver et al. 1997) and water *ad libitum*. The diet was freshly prepared weekly to prevent the fat from becoming rancid.

Following tumor establishment (14 days, tumor volumes ~ 300 mm^3), mice were stratified by body weight and tumor volume and randomly assigned to one of four groups (n=21 per group): (i) control group ii) exercise only, (iii) doxorubicin only, or (iv) exercise + doxorubicin. Doxorubicin (Adriamycin hydrochloride, Sigma Aldrich Canada Ltd, Oakville, ON) was administered via weekly intravenous lateral tail vein injections at 4mg/kg for 8 weeks. Injection sites were rotated to minimize local tissue irritation and injury. Exercise groups performed progressive treadmill running up to 18m/min at 0% grade for 45 min, 5d.wk for 8 weeks. Exercise training began at 10m/min, 0% grade, for 10 minutes for 5 days/week in weeks 1 and 2 and was systematically increased until the desired exercise protocol was achieved. This training intensity corresponds to approximately 70-75% of murine maximal oxygen uptake (Fernando, Bonen et al. 1993). Electrical stimulation was not used to encourage the animals to run. To ensure similar physical and social environments, a second treadmill was used as a sham exercise for the non-exercising groups (Thompson 1997). Tumor volume and body weight were measured twice weekly and animals were monitored continuously for the entire duration of exercise. Animal care was approved and in accordance with the Institutional Animal Care and Use Guidelines at the Cross Cancer Institute, Edmonton, Canada.

Mice were sacrificed when tumor volume reached 1100mm^3 as required by institutional guidelines or 48 hours following the final exercise training session. The primary endpoint was

tumor growth delay, calculated as the number of days for each individual tumor to reach 1100mm^3. Tumor growth delay survival curves were analyzed using the Cox model for pairwise comparisons and relative risk estimates generated with 95% confidence intervals. The logrank test was used for the overall group comparison. Changes in body weight were analyzed using independent samples t-tests. Two-tailed tests were used for the analysis with a $p<0.05$ considered significant.

Results and Discussion

Tumor growth delay and between group comparisons are presented in Table 1. Tumor growth delay was significantly prolonged in the doxorubicin only and exercise + doxorubicin groups compared to the exercise only and control groups. There was no significant difference between doxorubicin only and exercise + doxorubicin groups or exercise only and control groups. At 45 days, Kaplan-Meier estimates indicated a 35% (95% CI=17% to 54%) survival rate for the doxorubicin only mice compared to 20% (95% CI=7% to 33 %) in the exercise + doxorubicin group, 16% (95% CI=2% to 31%) in the exercise only group and 0% in the control group (Figure 1). Body weight did not significantly change over the course of experiment in any group (Figure 2). All mice achieved the designated exercise protocol.

The American Cancer Society recently published guidelines recommending that all cancer patients be encouraged to exercise during chemotherapy. These recommendations are primarily based on the promising preliminary evidence of the effects of exercise on maintaining or enhancing QoL (Brown, Byers et al. 2003). As such, studies investigating the potential interaction between exercise and chemotherapy efficacy are essential to the interpretation and acceptance of exercise as a modifier of QoL. As expected, groups that received doxorubicin had significantly prolonged tumor growth delay than groups who did not receive cytotoxic therapy. However, the key finding of this investigation was that moderate intensity treadmill running did not significantly modulate doxorubicin-induced tumor growth delay in MDA-MB-231 breast carcinoma xenografts.

A considerable number of exercise trials and preclinical studies in oncology have provided indirect evidence of the potential biologic mechanisms that may underlie the complex and multifaceted interaction between exercise, the tumor, and the antineoplastic effects of anthracycline-based chemotherapy. These biologic mechanisms include, but are not limited to exercise-modulated changes in hormonal (McTiernan, Tworoger et al. 2004) and metabolic profile (Fairey, Courneya et al. 2003), nitric oxide mediated peripheral blood flow (Hambrecht,

Hilbrich et al. 2000; Hambrecht, Wolf et al. 2000), angiogenesis (Kraus, Stallings et al. 2004; Waters, Rotevatn et al. 2004), endogenous antioxidant expression (Ji 2002), and pharmacokinetic profile of agents (van Baak 1990). However, the present findings provide preliminary evidence that an exercise training program reflective of national exercise guidelines for cancer prevention and cardiovascular health (Pate, Pratt et al. 1995) in humans may not sufficiently perturb these pathways to interact with the anticancer effects of doxorubicin on breast carcinoma xenografts.

Several prior preclinical reports have demonstrated an inhibitory effect of exercise training on spontaneous and chemically-induced tumor growth, metastatic progression and microvessel density without concurrent chemotherapy (Baracos 1989; Thompson 1994; Thompson, Westerlind et al. 1995; Westerlind, McCarty et al. 2003; Zielinski, Muenchow et al. 2004). However, almost all of these studies have examined the effects of exhaustive treadmill running at approximately 80-90% of maximal oxygen consumption (VO_{2max}). These previous findings in combination with the present results suggest that exercise training needs to be performed at a high intensity to activate tumor modulating pathways (>70% VO_{2max}) (Thompson 1994). Support for this notion is provided by the fact that the tumor growth curves for the exercise only and control groups were essentially identical in the present study (see Figure 1). Of course, from a clinical perspective, it is unlikely that many breast cancer patients undergoing cytotoxic therapy will be able or willing to exercise at 80-90% VO_{2max}. Thus, further research is required to investigate if a dose-response relationship exists between exercise and cancer progression during concurrent antineoplastic therapy.

Obviously, several important differences exist between our mouse model of breast cancer and breast cancer patients in the clinical setting. First, mice in the present study were only a few weeks post weaning and thus, immature. Second, hormonal and immune profile of the groups is not equivalent. This may be particularly important given the potential biologic interaction between exercise, immune/hormonal profile and breast cancer prognosis (28).

Finally, our breast cancer cell-line was implanted subcutaneously rather than at the relevant orthotopic site (i.e, mammary pad). Thus, caution must be taken when extrapolating the present results to women undergoing cytotoxic therapy for early-stage or metastatic breast cancer.

To summarize, the present results suggest that exercise training does not significantly modulate the antitumor efficacy of doxorubicin in breast cancer xenografts. Further studies investigating the effects of exercise on the cytotoxic effects of chemotherapy and other conventional agents in the clinical setting are warranted. Such studies are essential to fully understand the safety and application of exercise as a supportive intervention in cancer control.

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Table 1. Tumor growth delay of athymic female mice implanted with MDA-MB-231 breast carcinoma xenografts after treatment with doxorubicin (4mg/kg every 7 days), exercise training (18m/min, 0% grade, 45 mins, 5d.wk for 8 weeks), doxorubicin plus exercise or no intervention control.

	Median tumor growth delay (d)		RR (95% CI)	*P*-Level
Group	Control	Exercise		
No Doxorubicin	25	25	0.85 (.41-1.7)	0.65
Doxorubicin	42	36	1.44 (.69-3.0)	0.33
RR (95% CI)	0.38 (.15-.76)	0.54 (.27-1.1)		
P-Level	0.0084	0.080		

Tumor growth delay calculated as the number of days for each individual tumor to reach 1100mm^3. Tumor growth delay survival curves were analyzed using the Cox model for pairwise comparisons and relative risk (RR) estimates generated with 95% confidence intervals (CI).

Figure 1 Legend

Survival curves of athymic female mice implanted with MDA-MB-231 breast carcinoma xenografts. All animals were subcutaneously implanted with MDA-MB-231 breast carcinoma cells (5 x 10^6) in the right flank. Following tumor establishment (14 days, tumor volume ~ 300 mm^3), mice were stratified by body weight and tumor volume and randomly assigned to receive doxorubicin (4mg/kg every 7 days), exercise training (18m/min, 0% grade, 45 mins, 5d.wk for 8 weeks), doxorubicin + exercise or no intervention control. Tumor volume and body weight were measured twice weekly. Tumor growth delay was significantly prolonged in the doxorubicin only and exercise + doxorubicin groups compared with the exercise only and control groups (overall Log Rank p=0.015). There was no significant difference between doxorubicin only and exercise + doxorubicin groups or exercise only and control groups. At 45 days, Kaplan-Meier estimates indicated a 35% (95% CI=17% to 54%) survival rate for the doxorubicin only mice compared with 20% (95% CI=7% to 33%) in the doxorubicin + exercise group, 16% (95% CI=2% to 31%) in the exercise only group and 0% in the control group.

Figure 2 Legend

Mean body weights (grams) of athymic female mice implanted with MDA-MB-231 breast carcinoma xenografts. All animals were subcutaneously implanted with MDA-MB-231 breast carcinoma cells (5 x 10^6) in the right flank. Following tumor establishment (14 days, tumor volume ~ 300 mm^3), mice were stratified by body weight and tumor volume and randomly assigned to receive doxorubicin (4mg/kg every 7 days), exercise training (18m/min, 0% grade, 45 mins, 5d.wk for 8 weeks), doxorubicin + exercise or no intervention control. Tumor volume and body weight were measured twice weekly. There were no significant differences in body weight between the groups over the course of the study (p>0.05).